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The Nymphal-Adult Molt of the Silverleaf Whitefly (*Bemisia argentifolii*): Timing, Regulation, and Progress

Dale B. Gelman,[1]* Michael B. Blackburn,[1] Jing S. Hu,[1] and Dan Gerling[2]

The developmental progress of silverleaf whitefly (*Bemisia argentifolii)* 3rd instars and 4th instar/pharate adults was monitored using a tracking system that had been designed to identify synchronous individuals in another species of whitefly, the greenhouse whitefly, *Trialeurodes vaporariorum*. When reared on greenbean under conditions of LD 16:8 and a temperature of 26 ± 2°C, the body depth of 3rd instar SLWFs increased from approximately 0.04 mm (Stage 2) to 0.175–0.2 mm (Stage 7–8) and the body depth of the 4th instar increased from approximately 0.1 mm (Stage 1) to 0.25–0.30 mm (Stage 4–5). The durations of the 3rd instar and the 4th instar/pharate adult were approximately 3 and 7 days, respectively. Examination of coronal sections of 4th instars revealed that adult eye and wing development are initiated during Stage 6, the stage in which an external examination showed that the eye has begun to undergo pigment diffusion. Ecdysteroid titers peaked at approximately 400 fg/μg protein during stages 4 through 6A of the 4th instar, i.e., just prior to and upon the initiation of the pharate adult stage. Although adult development is initiated later in the SLWF than in the GHWF (adult eye and wing development begin in Stages 4 and 5, respectively, in GHWFs), the same rapidity of metamorphosis is observed in both species. Within approximately 24 h, the simple bi-layered wing bud developed into a deeply folded wing of nearly adult proportions and within an additional 12–24 h, the nymphal eye and wing bud had been replaced by the well-differentiated eye and wing of the adult whitefly. Our study is the first to describe the regulation, timing, and progress of the nymphal-adult molt and of the structural changes that accompany nymphal-adult metamorphosis in the SLWF. Arch. Insect Biochem. Physiol. 51:67–79, 2002. Published 2002 Wiley-Liss, Inc.†

KEYWORDS: silverleaf whitefly; staging system; developmental markers; ecdysteroids; molting; metamorphosis; morphology

INTRODUCTION

Bemisia argentifolii, the silverleaf whitefly causes hundreds of millions of dollars in crop losses each year (Heinz, 1996; Henneberry et al., 1997). Polyphagous in nature, the SLWF attacks plants by feeding on phloem, transmitting destructive pathogenic viruses and producing honeydew, a sweet, sticky substance that supports the growth of sooty mold. This black mold interferes with light transmission to chloroplasts and often causes harvesting equipment to malfunction. Reports of pesticide resistance in whiteflies (Horowitz and Ishaaya, 1995; Cahill et al., 1996a; 1996b) and concern for environmental safety have made the reduction of pesticide usage a primary goal for agriculture and has resulted in increased emphasis on the use of cost-effective biological control strategies and bio-

[1]Insect Biocontrol Laboratory, USDA, ARS, PSI, Beltsville, Maryland

[2]Department of Zoology, Tel Aviv University, Raqmat Aviv, Israel

Abbreviations used: ANOVA = one way analysis of variance; EIA = enzyme immunoassay; GHWF = greenhouse whitefly; SLWF = silverleaf whitefly; 20E = 20-hydroxyecdysone.

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*Correspondence to: Dr. Dale B. Gelman, USDA, ARS, IBL, Rm. 214, Bldg. 011A, Beltsville, MD 20705. E-mail: gelmand@ba.ars.usda.gov

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pesticide application in IPM programs. The development of insect-specific biopesticides such as juvenile hormone mimics (e.g., methoprene), molting hormone agonists [e.g., tebufenozide (RH5592)], and chitin synthesis inhibitors (e.g., diflubenzuron) was dependent upon the elucidation of the regulation of insect physiological and biochemical processes including molting, metamorphosis, cuticle synthesis, and egg production (as reviewed in Beckage, 2000). There is a serious lack of information concerning the regulation of physiological processes in whiteflies, information that is important for the development of new, cost-effective biopesticides, and that also will contribute to the development of artificial rearing systems for parasitoids that attack whiteflies. Previously, we described a precise staging system for identifying physiologically synchronous GHWFs (*Trialeurodes vaporariorum*), discussed the structural changes (internal and external) that accompany the molt from the GHWF nymph to the adult, and tracked molting hormone fluctuations in last instar/pharate adult GHWFs (Gelman et al., 2002). Here we use the staging system developed for the GHWF to identify physiologically synchronous SLWFs, to describe structural changes that occur during SLWF adult development and to titer the molting hormone in developing 4th instars and pharate adult SLWFs. Similarities and differences between the two species of whiteflies are detailed.

MATERIALS AND METHODS

Chemicals

Twenty-hydroxyecdysone was purchased from Sigma (St. Louis, MO). Tim Kingan (University of California at Riverside) provided the ecdysone antiserum and the peroxidase-labeled ecdysone conjugate used in the enzyme immunoassay (EIA). The antiserum has a high affinity for ecdysone, 20E, 3-dehydroecdysone, 20,26-dihydroxyecdysone, 26-hydroxyecdysone, and makisterone A (Kingan, 1989, personal communication). The goat-antirabbit IgG and the enzyme substrate, 3,3′,5,5′-tetramethylbenzidine (TMB), were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and American Qualex (San Clemente, CA), respectively, and the Coomassie Plus-200 Protein Assay Reagent was obtained from Pierce (Rockford, IL).

Insect Rearing

SLWFs were maintained in climate-controlled insect growth chambers/incubators (26 ± 2°C, light:dark regimen of L:D 16:8 and relative humidity of 60–80%) at the Insect Biocontrol Laboratory, Beltsville, MD. A variety of plants including greenbean, eggplant, tomato, poinsettia, and cotton served as hosts for the SLWF colony. For experiments, leaves of greenbean cuttings were infested with SLWFs. Cuttings were generated by removing leaves from greenbean plants at the junction of the petiole and stem and placing them in 60-ml tubes containing water and 1% Miragro (Miracle-Gro Products, Inc., Port Washington, NY). The leaves were infested with SLWFs by placing cuttings in a mesh bag containing between 100 and 300 whiteflies for approximately 18 h. After infestation, adult whiteflies were removed and leaves were returned to the incubator.

Insect Staging

Identification of instar. The ranges for length and width for each of the four SLWF instars (for these studies, 1st, 2nd, 3rd, and 4th instar, respectively, = the nymph from hatch to 1st ecdysis, the nymph from 1st to 2nd ecdysis, the nymph from 2nd to 3rd ecdysis, and the nymph from 3rd ecdysis to the initiation of adult development) were determined as described previously for GHWF (Gelman et al., 2002). Briefly, a small pen mark was placed next to each 1st instar whitefly that was to be tracked, and a locator map was generated. Whiteflies were examined daily between 11:00 A.M. and 2:00 P.M. until adult emergence or until mortality was observed. Length and width were recorded. Since whitefly larvae do not grow in length or width during a given instar (Hargreaves, 1915), a sudden increase in both length and width indicated that a molt had occurred. Typically, whitefly exuviae were present in the vicinity of newly molted nymphs.

Staging 3rd and 4th instar SLWFs. Because whiteflies do not develop synchronously, it was necessary to stage them prior to use in experimental procedures. Newly molted whitefly larvae are very flat and increase in depth (dorso-ventral axis) during the instar (Hargreaves, 1915). This increase in depth (measured at the point where the body is the thickest) served as the basis for staging 3rd instars (Gelman et al., 2002). The depth of Stage-1 3rd instars ranges from 0.025 to 0.035 mm and the depth of Stage-8 3rd instars ranges from 0.19 to 0.20 mm. The values for Stages 2, 3, 4, 5, 6, and 7 are 0.050, 0.75, 0.1, 0.125, 0.150, 0.175 ± 0.01 mm, respectively (Gelman et al., 2002). Both increase in body depth and the development of the adult eye were used in staging 4th instars (Gelman et al., 2002). The median depth values for Stages -1, -2, -3, and -4 4th instars were 0.1, 0.15. 0.2, and 0.25, respectively. SLWF 4th instars whose depth was ≥ 0.27 mm were designated Stage 5. When the eye, which appears as a red pinpoint (~0.01 mm) during Stages 1 through 5 of the 4th instar, begins to exhibit pigment diffusion, the whitefly has reached its maximum depth, and the pharate adult stage has been initiated (Gelman et al., 2002). In order to monitor the synchrony of development during the 3rd instar and the 4th instar/pharate adult stage, late (plump larvae whose lateral bottom edges were slightly raised from the leaf surface) 2nd or 3rd instar whiteflies were identified either early in the morning (between 8:00 and 9:00 A.M.) or early in the evening (between 5:00 and 6:00 P.M). Whiteflies that were 2nd or 3rd instars in the morning and had molted to the 3rd or 4th instar, respectively, by early evening were considered to be 1/3 day old. Those that were 2nd or 3rd instars in the evening and had molted to the 3rd or 4th instar, respectively, by the next morning were considered to be 2/3 day old. Since the actual time of the molt could not be identified, ages were approximations. A locator map was generated so that whitefly maturation could be tracked. At least 20 3rd instar whiteflies were examined at each third of a day and body depth was recorded until all 3rd instars had molted to the 4th instar. Data were collected from at least 2 different cohorts of SLWFs. Body depth of 4th instars and the appearance of the pharate adult eye [its change from a small, intense red dot (Stages 1–5), to a diffuse dot (Stage 6), to a light red adult eye (Stage 7), to a medium bipartite red (Stage 8) and finally dark red or red-black bipartite adult eye (Stage 9)] were recorded until adult emergence or death was observed. Photographs of Stages 2, 4, 6, 7, 8, and 9 of the SLWF are shown in Figure 1. The subdivision of Stage 6 into Stages 6A, 6B, and 6C was based on the amount of pigment diffusion observed. SLWFs in which diffusion was limited to the anterio-medial portion of the eye were placed in Stage 6A. When the diffused pigment had begun to radiate in all directions, the whitefly had progressed to Stage 6B, and when the diffused pigment created a circle whose diameter was 0.05 mm, the SLWF had reached Stage 6C. For 3rd instars, results are presented as percent of each stage observed on a given third of a day. However, since the duration of the 4th instar/pharate adult was between 6 and 7 days, results are presented as percent of each stage on a given day rather than on a given third of a day.

Sampling and Extraction of *B. argentifolii* for Ecdysteroid Determination

Staged 4th instar/pharate adult SLWFs were removed from the leaf and ecdysteroid extracts were prepared as described previously (Gelman et al., 2002). Briefly whiteflies were homogenized in 1.5 ml microcentrifuge tubes that contained 75 µl of ice cold 75% aqueous methanol. After centrifugation for 5 min at 4°C and 14,000*g*, supernatants were transferred to 6 × 50 mm borosilicate glass tubes that were placed in crushed ice. Washes of precipitates were added to their respective tubes and tubes were stored in the freezer at –10°C. An EIA (Kingan and Adams, 2000; as described in Gelman et al., 2002) was used to determine the ecdysteroid content of each sample. The range of the assay is 500 to 40,000 fg. The fg ecdysteroid

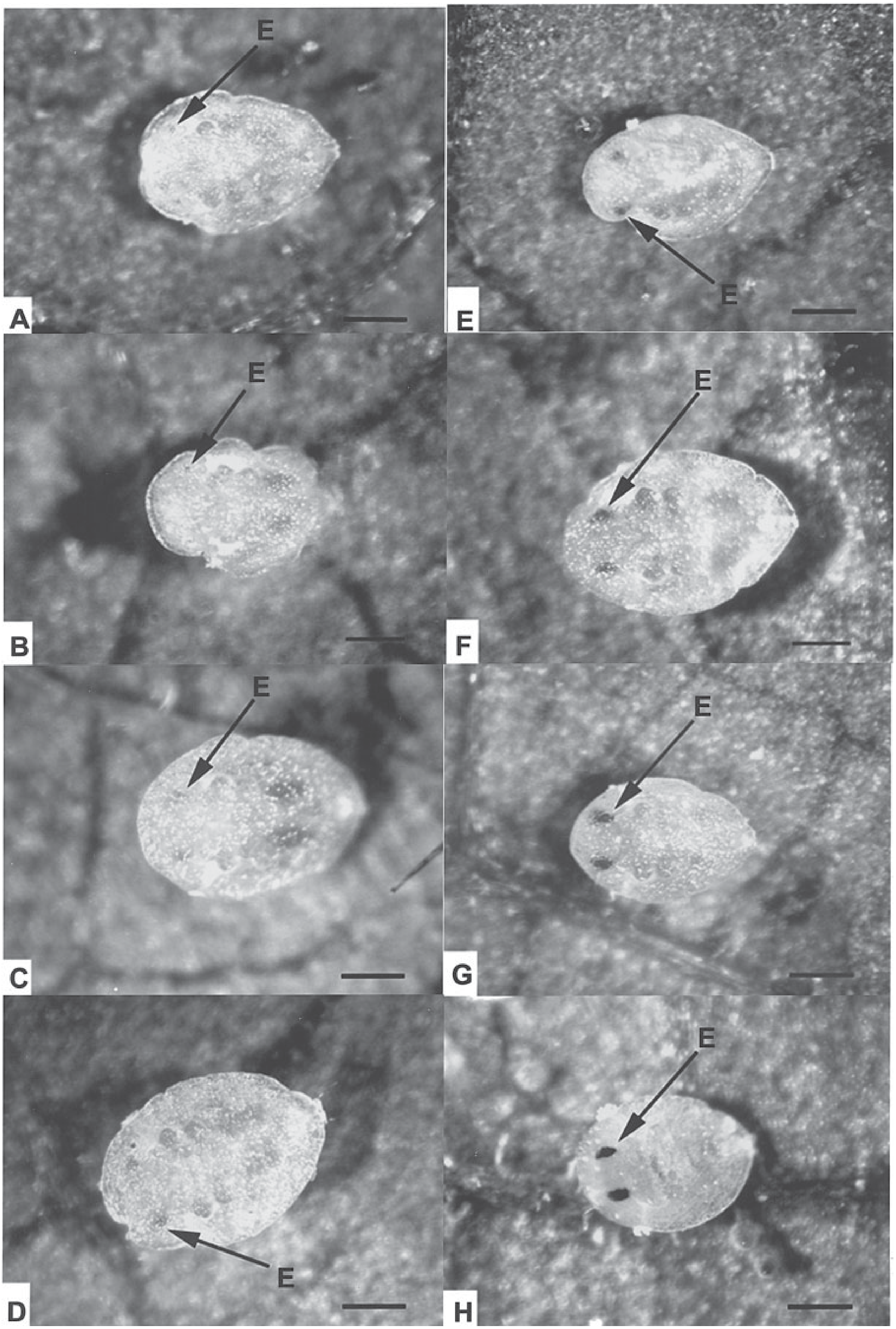

Figure 1.

present in each sample was calculated from a standard curve (semi-log plot with fg ecdysteroid plotted on the log scale) using the data analysis program, "Softmax." Since 20E was used as the standard, results are expressed as fg 20E equivalents/SLWF or /µg protein. In order to remove the contribution of the gut contents to whole body ecdysteroid titers, ecdysteroid content of filter chamber/midgut complexes, sometimes with hindgut attached (for anatomy, see Weber, 1935), was subtracted from whole-body ecdysteroid titers (Gelman et al., 2002a,b).

Protein Determination of Extracts of SLWFs

Extracts were prepared by homogenizing between 2 and 20 staged whiteflies in 1.5-ml microcentrifuge tubes containing 75 µl of sodium acetate buffer (0.05 M, pH = 5.3). Homogenates were sonicated in a water bath for 30–60 sec and then centrifuged for 10 min at 4°C and 14,000*g*. Aliquots were transferred to wells of a 96-well microtiter plate, and soluble protein was determined using the Pierce Coomassie Plus Protein Assay (Microwell Plate Version, micro protocol). Standards of 1–25 µg/ml were prepared from bovine serum albumin. Absorbance was measured at λ = 595 nm using an ELISA plate reader. The protein content of each sample was determined from the standard curve (log-log plot), again using the "Softmax" Data Analysis program.

Statistical Analysis

The ecdysteroid/protein titer data was analyzed using ANOVA. When F-tests were significant, the Fisher's Least Significant Difference (LSD) Comparison of Means Test was used to analyze for significant differences among the various groups (α = 0.05).

Fig. 1. The external appearance of the eye in Stage 2 (**A**), 4 (**B**), 6A (**C**), 6B (**D**), 6C (**E**), 7 (**F**), 8 (**G**), and 9 (**H**) 4th instar/pharate adult SLWFs. For descriptions, see Staging 3rd and 4th Instar SLWFs. The length of the bar = 0.2 mm.

Histological Methods

Whitefly nymphs were fixed in Carnoy's Formula 2 (Davenport, 1960) for 2–3 h, rinsed with absolute ethanol, stained for 30 min with 1% eosin b in absolute ethanol, and then washed with absolute ethanol to remove excess free eosin. The eosin stain conveyed a pink color to the whiteflies so that they would be easily visible during the embedding process. Dehydrated nymphs were passed through 4 changes of xylene before being placed in paraffin (Paraplast Xtra) at 60°C for 14–16 h. The whiteflies were then transferred to embedding molds that contained fresh paraffin and chilled rapidly in ice water.

A rotary microtome was used to prepare 5-µm sections of the embedded nymphs. Sections were relaxed on water at 40°C, mounted on egg albumin-coated slides, dried, and placed horizontally in a drying oven at 40°C overnight.

Deparaffinization was accomplished by passing the sections through 3 changes of xylene, followed by 2 changes of absolute ethanol. Sections were rehydrated by transfer through a series of aqueous ethanol solutions (95, 90, 70, and 50%) and then stained with Weigert's iron hematoxylin followed by Casson's trichrome (Kiernan, 1990).

RESULTS

Length and Width Measurements of the Four Instars of the SLWF

The mean length and width of the four instars of the SLWF increased in a step-wise fashion (Fig. 2). The ranges of adjacent instars, while not overlapping were not separated by large margins. Thus, the maximum length and width observed for 1st instars were only 0.1 mm less than the minimum length and width for 2nd instars. Similarly, maximum values for length and width of 2nd instars were only 0.04 and 0.02 mm less than values for minimum length and width, respectively, for 3rd instars.

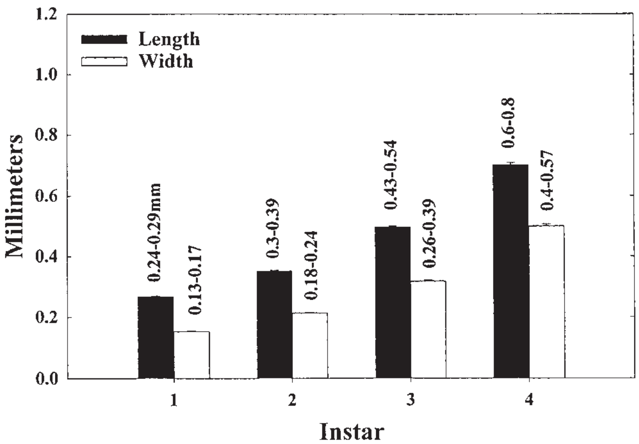

Fig. 2. Mean length and width of the 4 instars of the SLWF. SLWFs were reared on greenbean under conditions of 26 ± 2°C, a light:dark regimen of L:D 16:8 and a relative humidity of 60–80%. Individual whiteflies were marked and examined daily and an optical micrometer was used to determine the length and width measurements of each whitefly. Data were recorded until the whitefly emerged or mortality was observed. A jump in both length and width indicated that a molt had occurred. Each value represents the mean ± S. E. for at least 40 separate determinations. The ranges of length and width measurements are provided above each bar.

Stage as Related to Day Post-Molt to the 3rd and 4th Instar

Typically, under the rearing conditions used, the duration of the 3rd instar was between 2 and 3 days with approximately 70% of 3rd instars molting to the 4th instar by day 2 and 1/3, and 100% molting by Day 3 (Table 1). Table 1 also shows the progress of development of 3rd instars as a function of day post-molt. By day 1, the majority (95%) of 3rd instars had progressed to Stage 4 or 5, and by day 2, the majority (75%) had reached Stage 6 or 7.

The duration of the 4th instar was between 6 and 8 days with 100% emergence observed by day 8 post-molt to the 4th instar (Table 2). On days 0 and 1 post-molt, the population was relatively synchronous; most 4th instars were at Stage 1. By Day 3, 5 stages were observed with 18% having reached Stage 7. One day later, on day 4, a few 4th instars (5%) had already reached Stage 9 even though a similar percentage were at Stage 3. However, on Day 5, the population was fairly synchronous again; only two stages were observed, 8 (9%) and 9 (91%).

TABLE 1. Stage as Related to Day of the 3rd Instar of the SLWF*

	Stage (%)								
Day	1	2	3	4	5	6	7	8	M
1/6		70	30						
1/3		47	37	16					
2/3			36	64					
1			5	50	45				
1 1/3					67	33			
1 2/3				10	50	40			
2					25	45	30		
2 1/3						12	18	2	68
2 2/3						15	16	0	69
3									100

*Reared on greenbean at 26 ± 2°C, L:D 16:8. Day 1/6 = whiteflies examined within 4 h of the molt. At least 20 SLWFs were examined at each fraction of a day, i.e., 1/6, 1/3, 2/3, etc. M = molt to the 4th instar.

Soluble Protein Content of 3rd and 4th Instar SLWFs

Hormone/metabolite concentration is usually expressed in units/μl hemolymph. However, since the collection of hemolymph was impractical, in order to correct for differences in body size, ecdysteroid content was expressed as fg/μg soluble protein as well as fg/whitefly. Mean amounts of soluble protein for each stage of the 3rd and 4th instar are provided in Table 3. Protein content increased during the 3rd instar and reached its highest level in Stages 6 through 8. For 4th instars, protein content also increased during the instar, but peaked by Stage 4. Content was similar from Stages

TABLE 2. Stage as Related to Day of the 4th Instar of the SLWF*

	Stage (%)									
Day	1	2	3	4	5	6	7	8	9	E
0	100									
1	88	12								
2	11	57	32							
3			18	18	6	41	18			
4			6	1	0	14	26	48	5	
5								9	91	
6									43	57
7									31	69
8										100

*Reared on greenbean at 26 ± 2°C, L:D 16:8. Day 0 = the day of the molt to the 4th instar; whiteflies were examined within 8 h of the molt. Between 20 and 60 SLWFs were examined on each day. For this experiment, Stage 6 SLWFs were not subdivided into Stages 6A, B, and C. E = adult emergence.

TABLE 3. Soluble Protein Content of 3rd and 4th Instar SLWFs[†]

	μg Protein/SLWF*			
Stage	3rd Instar		4th Instar	
1			0.854 ± 0.146	(E)
2	0.094 ± 0.011	(e)	1.548 ± 0.130	(DE)
3	0.238 ± 0.033	(de)	2.068 ± 0.320	(CD)
4	0.402 ± 0.012	(cd)	3.017 ± 0.893	(ABC)
5	0.471 ± 0.036	(bc)	3.022 ± 0.240	(AB)
6	0.484 ± 0.031	(abc)	2.801 ± 0.408	(ABC)
7	0.633 ± 0.094	(ab)	2.591 ± 0.193	(BC)
8	0.680 ± 0.141	(a)	2.686 ± 0.338	(ABC)
9	—		3.517 ± 0.319	(A)

[†]Each value represents the mean ± S.E. for at least 3 separate determinations. Each sample contained between 3 and 20 SLWFs.
*Means followed by the same letter(s) are not significantly different.

4 to 9, although for Stage 9, protein concentration was significantly higher than for Stage 7.

Fluctuations in Ecdysteroid Concentration During the Development of 4th Instar/Pharate Adult SLWFs

Ecdysteroid content of the filter chamber/gut complexes removed from Stages 2–6 GHWFs and SLWFs whiteflies is not significantly different (Gelman et al. 2002a,b) and in the SLWF the mean is 67 fg/gut complex. This value was subtracted from whole body whitefly titers in order to eliminate the contribution of phytoecdysteroids in the gut. Whether expressed as fg 20E equivalents/whitefly or /μg protein, SLWF ecdysteroid titers increased during Stages 1 through 4, plateaued between stages 4 and 6A, and then decreased during the rest of the instar (Fig. 3).

Horizontal Sections of 4th Instar SLWFs

Morphological changes of the eye and wing (Fig. 4) were the most reliable indicators of the onset of adult development. Nomenclature of structures that compose the eye is after Snodgrass (1935). The eyes of Stage-3 (not shown), -4 (Fig. 4A), and -5 (Fig. 4C) nymphs were similar; the retina was undeveloped and the corneagenous cells that ultimately give rise to the cuticular cornea remained indistinct during these stages. At Stage 6,

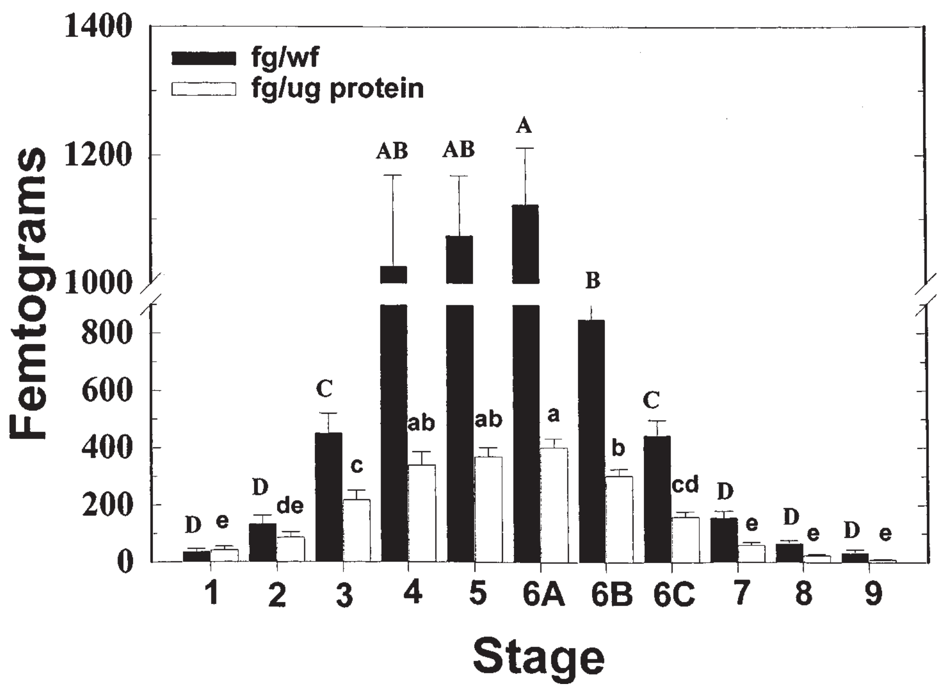

Fig. 3. Whole body ecdysteroid fluctuations during the 4th instar/pharate adult. Whiteflies were extracted in aqueous methanol and titers were determined using an ecdysteroid EIA as described in Materials and Methods. Titers are expressed as fg 20E equivalents per whitefly and per μg protein. The contribution of the filter chamber/gut complex was subtracted from each value prior to the determination of the mean. Each bar represents the mean ± S.E. of at least 5 separate determinations. Means having the same letter designation were not significantly different.

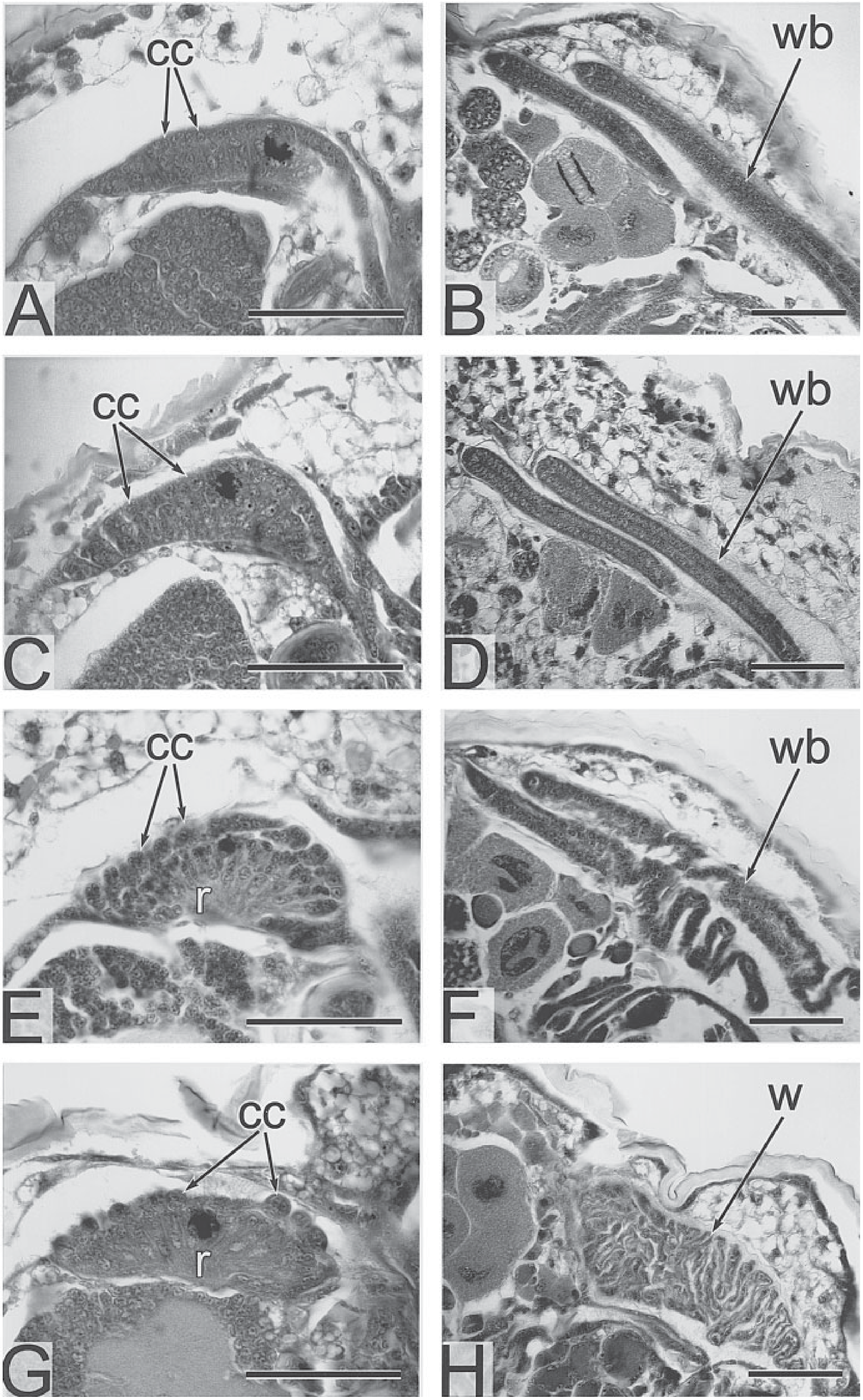

Figure 4.

the corneagenous cells began to change taking on a more rounded shape and the retinal cells had begun to lengthen axially (Fig. 4E). It is in this stage that the diffusion of the eye pigment can first be observed externally. In the eye of a Stage-7 SLWF, the corneagenous cells have undergone further development and the retinal cells have lengthened substantially (Fig. 4G). By Stage 8 (not illustrated), it appeared that the adult eye was fully developed.

Wing buds were relatively simple in structure in Stage-3 (not shown), -4 (Fig. 4B), and -5 (Fig. 4D) 4th instars. Coronal sections (longitudinal sections that divided the body into dorsal and ventral halves) revealed that through Stage 5, the wing buds were composed of a distinct bilayer of columnar epithelial cells. In Stage-6 4th instars, the wing buds began to undergo convolution (Fig. 4F). The degree of folding increased between Stages 6A and 6C. By Stage-7 (Fig. 4H), the wings had become highly convoluted and had expanded to nearly adult proportions. Spines could now be seen on the surface of the wings. Wings of Stage-8 4th instars (not shown) were similar in appearance to those of Stage-7 whiteflies, indicating that the period of explosive growth occurred during Stage 7, a stage of relatively short duration. Also apparent, was the rapid development of flight muscle in the thorax of Stage-7 insects.

DISCUSSION

A comparison of developmental rates, timing and progress of metamorphosis to the adult, and accompanying ecdysteroid titer fluctuations for the silverleaf and greenhouse whitefly revealed that there are important differences between the two species. When mean lengths and widths and ranges of lengths and widths for the 4 instars of greenhouse and silver leaf whiteflies reared on greenbean were compared, it was confirmed that GHWFs tend to be longer and wider than SLWFs during the first 3 instars (Hill, 1969). However, for 4th instars, both the mean length and the range for lengths of the two whitefly species were similar, and the mean width and range for widths were greater in the SLWF (range = 0.4–0.57 mm) than in the GHWF (range = 0.35–0.51 mm) (Gelman et al., 2002 for GHWF lengths and widths). Hill (1969) reported that when the two species of whiteflies were reared on tobacco, both mean length and mean width were greater for the SLWF. The appearance and size of a whitefly will vary depending on the identity of the host plant (Mound, 1963; Bethke et al., 1991; Rosell et al., 1995; Neal and Bentz, 1999), and, therefore, before generalizing, a comparison of other host plants should be undertaken.

Typically, only mean lengths and widths for a given instar reared on a particular host plant have been reported (Hill, 1969; Bethke et al., 1991). However, as in the GHWF, ranges for SLWF length and width of adjacent instars, while not overlapping, are quite close [in GHWF, they can overlap depending upon the identity of the host plant; (Gelman et al., 2002a)], and therefore, knowledge of the means of instar length and width would not be very helpful in distinguishing among instars. Measurements of length and/or width can be used for identifying instars (especially for 3rd and 4th instars), but it appears that the product of length $\times$ width is the best parameter for distinguishing between adjacent SLWF instars, especially between 1st and 2nd instars (Gelman et al., 2002b).

The system described for staging 3rd and 4th instar GHWFs (Gelman et al., 2002a) was very useful for staging these two instars of the SLWF. However, no Stage-1 3rd instar SLWFs were observed, even when nymphs were examined within 2 h of being observed as plump 2nd instars. The minimum depth recorded for 3rd instar SLWFs was 0.04 mm. Stage-5 4th instar whiteflies were far less numerous in populations of SLWF than in populations of GHWF. These results suggest that many

Fig. 4. Histological sections of Stage-4, -5, -6A, and -7 4th instar/pharate adult SLWFs. **A,C,E,G:** The eye margin (indicated by arrows) for Stages 4 through 7, respectively. The bar in the lower right of each photo is equal to 50 µm. **B,D,F,H:** Track wing development for Stages 4 through 7, respectively. The bar in the lower right of each photo is equal to 50 µm. cc = corneagenous cells, r = retina, wb = wing buds, w = wing. See Materials and Methods for histological methods.

SLWF 4th instars do not pass through Stage 5 before initiating adult development. Interestingly, ecdysteroid titers in Stage-4 and -5 SLWF 4th instars were approximately the same. The durations of the 3rd instar were similar for the two species of whiteflies, but the duration of 4th instar GHWFs was approximately 2 days longer than that of 4th instar SLWFs (Gelman et al., 2002b).

The nine stages described by Gelman et al. (2002a) for 4th instar/pharate adult GHWFs were also observed in maturing last instar/pharate adult SLWFs. Populations of 4th instar and/or pharate adult SLWFs were relatively synchronous on days 0–2 and 5–8 post-molt to the 4th instar. However, on days 3 and 4 post-molt, 5 and 6 different stages, respectively, were observed, stages that included premolt individuals as well as new, young, and mature pharate adult whiteflies. Nechols and Tauber (1977) described a staging system in which the 4th instar GHWF was divided into 3 stages, "early" (flattened, translucent to opaque-whitish, our Stage 1), "transitional" (expanded, wax-ensheathed, opaque-white with dorsal and lateral, waxy, spine-like processes, our Stages 2–5), and pharate adult ("transitional," but with red eyes and yellow body pigment of developing adult visible, our Stages 6–9, although Stages 6 and 7 do not exhibit a yellow-body color). For the SLWF as well as the GHWF, division of the last instar/pharate adult into only 3 stages would prevent the detection of important physiological/biological changes that occur during maturation.

Total ecysteroid titer was measured using an exceptionally sensitive EIA, the range of which was 500–40,000 fg 20E equivalents. Expressed as either fg/whitefly or fg/µg protein, ecdysteroid titers peaked between Stages 4 and 6A, just prior to and at the onset of pharate adult development (Fig. 3), as would be expected based on results reported for insects of other orders (reviewed by Riddiford and Truman, 1978; Smith, 1985). Since the ecdysone antibody binds to a number of different ecdysteroids, concentrations of individual ecdysteroids were not determined in these investigations. Therefore, results do not necessarily reflect fluctuations of physiologically-active ecdysteroid, typically 20E in almost all orders examined (reviewed by Smith, 1985), although makisterone A has been reported to be the molting hormone in some hemipterans and in honey bee pupae (Kelly et al., 1985; Feldlaufer et al., 1985). The homopteran molting hormone has not yet been characterized.

Ecdysteroid titers were three to four times greater in SLWF than in GHWF (Gelman et al., 2002a) 4th instar/pharate adults. When expressed as fg/whitefly, ecdysteroid peaked at approximately 350 fg in Stages 4 and 5 of 4th instar GHWFs as compared to approximately 1,100 fg in Stages 4–6A of 4th instar SLWFs. When expressed as fg/µg protein, ecdysteroid levels equaled approximately 100 and 400 fg during GHWF Stages 3–6 and SLWF Stages 4–6A, respectively. In the only other homopteran in which ecdysteroid titers have been tracked, the aphid, *Acyrthosiphon pisum*, ecdysteroid titers for 4th instars were determined for hemolymph extracts (Pennacchio et al., 1995), and, therefore, it is difficult to compare the absolute titers in the two homopteran species.

The first external sign of adult development (diffusion of the eye pigment) occurs in Stage-6 whiteflies. An examination of coronal sections of whole body preparations of SLWF also revealed that adult development is initiated at Stage 6. The rounding of the corneagenous cells of the eye and beginning invagination of the wing buds, changes associated with the initiation of adult development, were first observed in Stage-6 SLWFs. In contrast, these changes were detected earlier in GHWFs, in Stages 4 and 5, respectively. Interestingly, SLWF ecdysteroid titers dropped significantly between Stages 6A and 6B and between Stages 6B and 6C while ecdsyteroid titers of GHWFs dropped between Stages 5 and 6A and then remained at the same level through Stage 6C (unpublished results). Thus, the earlier initiation of the adult stage in the GHWF is accompanied by an earlier drop in ecdysteroid titer.

Insects usually cease to feed and hence to grow just prior to apolysis and do not reinitiate feeding until ecdysis to the next instar. Whiteflies, however, continue to feed throughout the 3rd and 4th instars and the pharate adult stage (Lie et al., 1996;

Costa, et al., 1999; Gelman et al., unpublished results). Observed increases in soluble protein content during the 3rd instar of the GHWF (Gelman et. al., 2002a) and SLWF are consistent with feeding behavior. Once the molt to the 4th instar has been initiated, increase in body mass does not level off, except between Stages 5 and 6 when ecdysteroid titers drop precipitously (Gelman et al., 2002b). However, in the 4th instar, despite continuous feeding in both greenhouse and silverleaf whiteflies, soluble protein content increases through Stage 4 (onset of adult development), remains relatively constant through Stage 8, and then increases in Stage 9, the latter increase being somewhat steeper in the GHWF than in the SLWF. Thus, during most of whitefly pharate adult development, soluble protein content remains stable even though all stages were observed to feed.

Although the initiation of adult development was observed to occur at an earlier stage in the GHWF (Stage 4) (Gelman et al., 2002a) than in the SLWF (Stage 6), once initiated, metamorphosis occurred at an explosive rate in both species. In less than 24 h, i.e., between Stages 6 and 7 of the SLWF, the simple bilayers that compose the wing (in Stage 6) become exceedingly invaginated, and the size of the wing approximates that observed for the adult whitefly. In little more than 24 h, between Stages 6 and 8, the relatively simple nymphal eye has developed into a well-differentiated, relatively complex, bipartite adult eye. In other insect orders, the initiation of apolysis and the deposition of new adult cuticle has been reported to exhibit an anterior to posterior gradient (Rembold et al., 1980; Riddiford, 1985; Gelman et al., 2000a). Although as in the GHWF, the exact time of apolysis and the deposition of adult cuticle could not be determined from histological sections, metamorphosis of the wing in the SLWF as in the GHWF also exhibited an anterior to posterior gradient with the anterior portion beginning to fold before the posterior portion. However, in contrast to the condition in the GHWF (Gelman et al., 2002a), the SLWF eye and wing initiate adult development at approximately the same time (i.e., in Stage 6).

In summary, the staging system described for tracking development in 3rd instar and 4th instar/pharate adult GHWFs was useful for monitoring maturation in SLWFs. Based on ecdysteroid titer determinations and histological examination, it was concluded that adult development is initiated during Stage 6, the stage in which external examination reveals that the eye pigment has become diffuse. Ecdysteroid titers peaked during Stages 4 through 6A, and in all Stage-6 whiteflies, the corneagenous cells of the eye had become more rounded and pronounced, and the cells that compose the wing buds began to multiply rapidly. Metamorphosis to the adult occurred at a very rapid rate. Within 1.5–2 days (by Stage 8), the wing and eye had reached adult proportions. These results complement those reported for GHWF and together they elucidate the regulation, timing, and progress of the nymphal-adult whitefly molt and of adult metamorphosis.

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